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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

008325

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

APR -5 1991

MEMORANDUM

SUBJECT: Naled - Acute Neurotoxicity Data, Submitted Under MRID No. 416307-01
EPA Registration Nos. 62499-14 and 59639-43

Chemical (Caswell) No.: 586
RD Record No.: S-388627
HED Project No.: 1-0464

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Registrant: Valent USA, Walnut Creek, CA/Chevron Chemical's Environmental Health Center (EHC), Richmond, CA

Request

Review and evaluate the following acute study, submitted as indicating possible neurotoxicity effects:

Acute Delayed Neurotoxicity Study with Naled Technical in the Domestic Hen, performed at the Huntington Research Centre (HRC), Cambridgeshire (UK), Final Report No. (HRC) CHR 33/90539, issued 30 July 1990 (EPA MRID No. 416307-01).

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TB Conclusions: CORE-MINIMUM DATA

- Acute (Oral) LD_{50} = 42 mg/kg
- At 42 mg/kg: Increased axonal degeneration
  : Depressed brain ChE activity

Attachment (DER)

Reviewed By: Irving Mauer, Ph.D., Geneticist 3-28-91
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief 4/3/91
Toxicology Branch I - IRS (H7509C)

DATA EVALUATION RECORD 008325

I. SUMMARY

MRID No.: 416307-01
ID Nos.: 62499-14 and 59639-43
RD Record No.: S-388627
Caswell No.: 586
Project No.: 1-0464

Study Type: (81-7) Acute Delayed Neurotoxicity - Hen

Chemical: Naled: 034401 (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate)

Synonyms: DIBROM

Sponsor: Valent

Testing Facility: Huntingdon Research Center, Cambridgeshire (UK)

Title of Report: Acute Delayed Neurotoxicity Study with Naled Technical in the Domestic Hen.

Authors: V.A. Redgrave, C. Gopinath, and A. Anderson

Study Number: CHR 33/90539

Date of Issue: July 30, 1990

TB Conclusions:

Oral LD_{50} = 42 (26-59) mg/kg

No signs of frank delayed neurotoxicity (locomotor ataxia; depressed NTE) at the LD_{50}, but increased histopathological evidence of axonal degeneration in spinal cord and peripheral nerves, and significantly depressed brain cholinesterase activity.

Classification (Core-Grade): CORE MINIMUM

II. DETAILED REVIEW

A. Test Material - Naled Technical

Description: Straw-colored liquid
Batch (Lot): NB-10198-41
Purity (%): 97.5 to 98.8
Solvent/Carrier/Diluent: 0.5% aqueous sodium carboxymethylcellulose (S-CMC)

B. Test Organism - Bird

Species: Domestic hen (Gallus gallus domesticus)
Strain: "Heavy hybrid, brown" (not otherwise specified as to strain)
Age: 8 to 14 months (adult)
Weights: Females (only): 1.8 to 2.6 kg (at start)
Source: Atkinson Bros., Peterborough (UK)

C. Study Design (Protocol) - This study was designed to assess the neurotoxicity potential of naled technical when administered by oral gavage to adult hens, according to a protocol (Appendix 9 of Final Report), based upon Agency FIFRA Test Guideline 81-7 (1984).

Statements of Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were both provided.

D. Procedures/Methods of Analysis - Following preliminary range-finding (replicate acute tests on separate days in two groups of two birds each administered test compound at doses up to 560 mg/kg), formal LD_{50} determinations were conducted in groups of 10 each at five dose levels of naled, in addition to vehicle (SCMC) controls.

For the evaluation of neurotoxicity, two sets of test groups (5 to 10 hens each) were dosed once with test article (or its vehicle as solvent control), or with tri-ortho-cresyl phosphate (TOCP, 500 mg/kg) as positive control. The first set of naled-treated (single dose level of 42 mg/kg) birds were injected intramuscularly with atropine sulphate and 2-PAM immediately prior to dosing, observed for 21 days, then re-dosed once again, followed by another 21-day observation period before sacrifice (study Day 42). TOCP-treated birds of set-one, however, were sacrificed at Day 21.

At postmortern, heads, spinal columns, and sciatic nerves were fixed in 10% neutral formalin, and sliced (7μ) for histopathology. Microtome sections were stained with H&E (for conventional examination), or with silver impregnation (for axons), or with solochrome cyanin (for myelin).

Neuropathological lesions were graded according to the following scheme (Addendum 2, page 117):

Grade	Lesion(s)	
0	None in tissue examined.	
I	No white matter abnormality detected.	
	AXON DEGENERATION	MYELIN ABNORMALITIES
II	Occasional (1-2/slide)	Rare
III	Minimal (5+ per slide)	Minimal (spheroids only)
IV	Moderate (diffuse to focal in "many" axons)	Moderate (presence of macrophages)
V	Severe (widespread)	Severe (widespread), with glial/Schwann cell responses

The second set were given CMC vehicle above, or administered naled at two dose levels (8 and 42 mg/kg), or 500 mg/kg TOCP, and sacrificed 24 hours later. Brains were divided in two, each half weighed, frozen (-20 °C) in cardice-hexane, and sampled for brain cholinesterase (AChE) and neurotoxic esterase (NTE) activities.

The following statistical methods were applied to the data:

1) For body weights - One-way ANOVA on means of weeks 1 to 3 and 4 to 6, followed by Student's "t" (F ratio significance level at 5%).

2) For biochemical parameters - One-way ANOVA, and Student's "t".

3) For histopathological gradings - Kruskall-Wallis analysis of ranked data, separately for brain (two sites), spinal cord (4 sites), and periphenal nerves (3 sites), followed by the nonparametric equivalent of Student's "t" (F-ratio significance levels, 5% and 1%).

E. Results - [See summary on following DER page.]

In the range-finder, no bird survived single doses above 100 mg/kg. Hence, 128 mg/kg was selected for the formal LD_{50} assay.

In the acute assay (Phase-I in DER Table-I), two CMC-controls died, as did 1, 4, 9, and 10 test hens gavaged at 8, 32, 64, and 128 mg/kg naled, respectively.

Subdued activity was noted in all test groups, unsteady gait at 16 mg/kg and above, and gasping and salivation at 32 mg/kg and above. The LD_{50} was calculated to be 42 mg/kg (with 26 to 59 mg/kg as CL). Sporadic decreases in body weight occurred in 8 and 64 mg/kg survivors during the week after dosing; increased body weights were recorded in all other test groups.

DER TABLE A: Neurotoxic Effects of Naled Technical in Domestic Hens[1]

Observation or Determination	PHASE-I (LD_{50}) Naled Dose (mg/kg)						PHASE-II (Neurotoxicity) Dose (mg/kg)		
							Naled		TOCP
	0 (10)[2]	8 (10)	16 (10)	32 (10)	64 (10)	128 (10)	0 (10)	42 (39)	500 (10)
Deaths (n)	2	1	0	4	9	10	2	4	0
Mean Body Weight(g):									
Week - 0	1907	1824	1949	1888	1956	1826	1957	1924	1961
- 1	1915	1804	2063	2016	(1870)	--	1960	1865	1984
- 2	1908	1804	2068	2070	(1965)	--	1989	1946	2033
- 3	--	--	--	--	--	--	1978	1908	1910
- 6	--	--	--	--	--	--	2021	1967	--
Clinical:									
"Subdued"	0	7	10	7	1	0	0	40	0
"Unsteady"	0	0	5	9	7	9	0	40	0
Salivation/ gasping	0	0	0	9	7	9	--	0	0
Locomotor ataxia	-	-	-	-	-	-	0	0	2
Histopathology (mean grade response):									
Brain (3 sites)							1.00	1.00	1.25
Spinal cord (4 sites)							1.25	1.50**	2.25
Peripheral nerve (3 sites)							1.17	1.00	2.00

1/ Extracted from individual animal data (Appendices 3 to 8 and Addendum 2), and Summary Tabulations (text Tables 1 to 9, and Addendum 3) of the Final Report.
2/ Number of animals examined.
**Significant, $p < 0.01$.

In the assessment of delayed neurotoxicity, (Phase II of the included DER-Table), 2/10 controls and 4/40 naled birds died during the study, and all test hens manifested clinical signs of neurotoxicity. Minor, nonsignificant changes were recorded in body weight throughout the 6 weeks of the study, but these were nonsignificant ($p = 0.33$ for week 2 weighing, and 0.17 at week 5 - - - Addendum 3, Report Tables S1 and S2, attached). Histopath gradings were lower than TOCP birds in both vehicle control and naled-treated birds ($p < 0.01$), but higher than the vehicle value among naled-treated birds (Summary in Addendum 3, Report Table S4, attached). Compared to background in concurrent CMC control birds (mostly Grade I and II), the majority of the 10 TOCP hens showed at least Grade III (and often more severe) responses in at least one level of spinal cord, and Grade II to III in peripheral nerves and/or brain. The less severe (but statistically significant, $p < 0.01$) changes in naled-treated hens nevertheless indicated to the investigators compound-induced axonal degeneration in the spinal cord (but not peripheral nerves).

Brain cholinesterase activity was markedly reduced in naled groups (mean 6.6 u mol/g min., $p < 0.01$) compared to CMC control (14.5 u mol/g/min), but brain NTE appeared to be unaffected, mean value of control = 1774, vs. 1772 in naled hens (Addendum 3, Report Table S3).

In summary, the investigators concluded that naled at an oral LD_{50} dose (42 mg/kg) did not produce frank signs of delayed neurotoxicity (such as locomoter ataxia and depressed NTE); however, based upon equivocally increased, but nevertheless consistent, histopathological lesions (Grade III axonal degeneration in spinal cords and peripheral nerves) compared to both concurrent vehicle controls as well as the laboratory's historical controls (provided as Addendum 4 in the Final Report), thus suggesting to them the possibility that this compound may exert "some neurotoxic effect."

F. **Conclusion:** Core-Minimum Data

Attachments (Data Tables from the Final Report)

ATTACHMENT - I

Data Tables

Naled toxicology review

Page _____ is not included in this copy.

Pages 10 through 16 are not included in this copy.

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___ Sales or other commercial/financial information

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